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Crystal Structure of LeuA from *Mycobacterium tuberculosis*, a Key Enzyme in Leucine Biosynthesis

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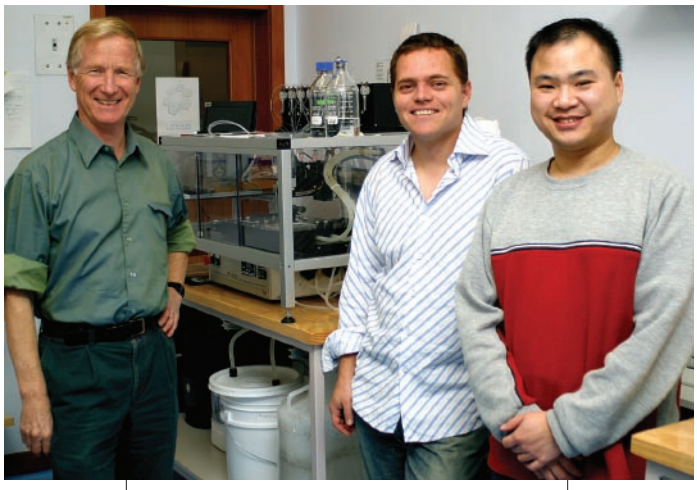
The leucine biosynthetic pathway is essential for the growth of Mycobacterium tuberculosis, and is a potential target for the design of new anti-tuberculosis drugs. We have used multiwavelength anomalous diffraction (MAD) phasing to determine the high-resolution crystal structure of α-isopropylmalate synthase, the product of the leuA gene. The structure reveals a tightly associated dimer comprised of an $(\alpha/\beta)_8$ barrel catalytic domain joined by flexibly-hinged linker domains to a C-terminal regulatory domain. The structure shows how the substrate, the leucine precursor α-ketoisovalerate, binds to an essential zinc atom in the catalytic domain, and a potential mechanism of feedback inhibition by leucine binding in the regulatory domain.

Tuberculosis (TB) is responsible for more deaths worldwide than any other infectious disease – more than two million each year, with an estimated one third of the world's population infected. The problem is compounded by the length of current treatments and by the ability of *Mycobacterium tuberculosis*, the causative agent, to survive in a dormant state inside activated macrophages in the lung for long periods of time, emerging many years later as active infections. Multi-drug resistance is also a growing problem. The genome sequence of *M. tuberculosis*, completed in 1998, has stimulated new approaches, including the formation of the TB Structural Genomics Consortium (http://www.doe-mbi.ucla.edu/TB/). Our goals, as part of this consortium, are to determine the three-dimensional structures of proteins that are potential new targets for TB drug development or that will help to understand TB biology.

The biosynthetic pathways for the branched-chain amino acids leucine, isoleucine and valine (i.e. the series of enzyme reactions that produce these amino acids) are essential for the survival of the TB organism. These pathways are not present in humans, indicating that inhibitors targeted against enzymes from them could be useful as anti-TB drugs. We chose the enzyme α-isopropylmalate synthase (α-IPMS), the product of the *leuA* gene, for structural analysis. This enzyme catalyzes the first step in leucine biosynthesis, in which an acetyl group is transferred from acetyl-coenzymeA to the substrate α-ketoisovalerate (α-KIV), the leucine precursor. The enzyme was known to be dimeric (2 x 70 kDa), metal ion-dependent, and subject to feedback inhibition by the end product leucine, but its three-dimensional structure was unknown.

The structure was solved using multiwavelength anomalous diffraction (MAD) data from a selenomethionine-substituted α-IPMS crystal, which was frozen and sent from New Zealand to beamline X8C (Fedex crystallography!) for data collection. The data quality was such that 70% of the 1288 residues in the dimer could be immediately traced and autobuilt. The structure was then completed and refined at 2.0 Å resolution. A spherical peak for a bound metal ion, marking the active site of each monomer, was



Authors (from left): Ted Baker, Chris Squire, and Nayden Koon

identified by a fluorescence scan as zinc. We also subsequently prepared complexes with α-KIV (substrate) and leucine (feedback inhibitor).

The α-IPMS monomer is folded into three domains (**Figure 1**), an $(\alpha/\beta)_8$ barrel catalytic domain, a helical linker domain and a C-terminal domain of novel fold. Two α-IPMS monomers form a domain-swapped dimer in which the linker domain of one monomer sits over the catalytic domain of the other. This domain swapping makes the active site almost completely enclosed, except for a tunnel that allows the entry of substrates. In the crystal structure, the substrate α-KIV is bound to the zinc (**Figure 2A),** and although acetyl-CoA is not present, two water molecules mark where the acetyl group would sit, adjacent to α-KIV. With this information we can propose a catalytic mechanism for α-IPMS, based on that of malate synthase, an enzyme that is evolutionarily unrelated but carries out a very similar reaction on a very similar substrate.

Several features make the α-IPMS structure suitable for drug development: the zinc ion as a template for inhibitor binding and surrounding invariant side chains inside the enclosed active site cavity. The structure also reveals an intriguing mechanism for feedback inhibition. Leucine binds in the regulatory C-terminal domain (**Figure 2B)** 70 Å away from the nearest active site, but this binding must be signalled *via* the flexible linker domain, which inserts two key residues into the active site.

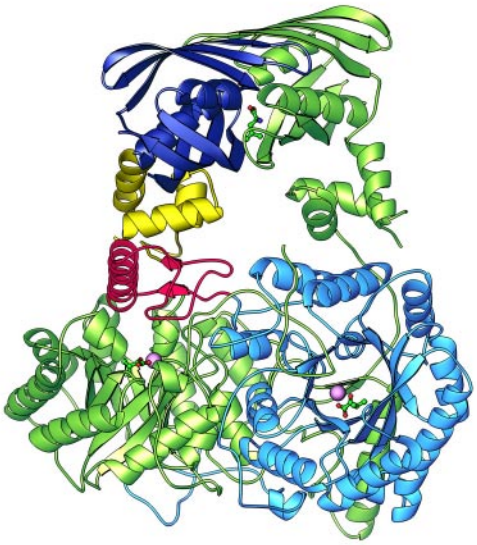


Figure 1. *Ribbon diagram of the LeuA dimer. One monomer is in green. The other is colored to show the three domains: the N-terminal catalytic domain (light blue), the central linker domain (shown as red and yellow subdomains), and the C-terminal regulatory domain (dark blue). The active site of each monomer is shown by the zinc atom (magenta sphere) and bound α-KIV substrate. Each active site is covered by helix α10 from the other monomer of the dimer. A leucine molecule is shown bound in the regulatory domain.*

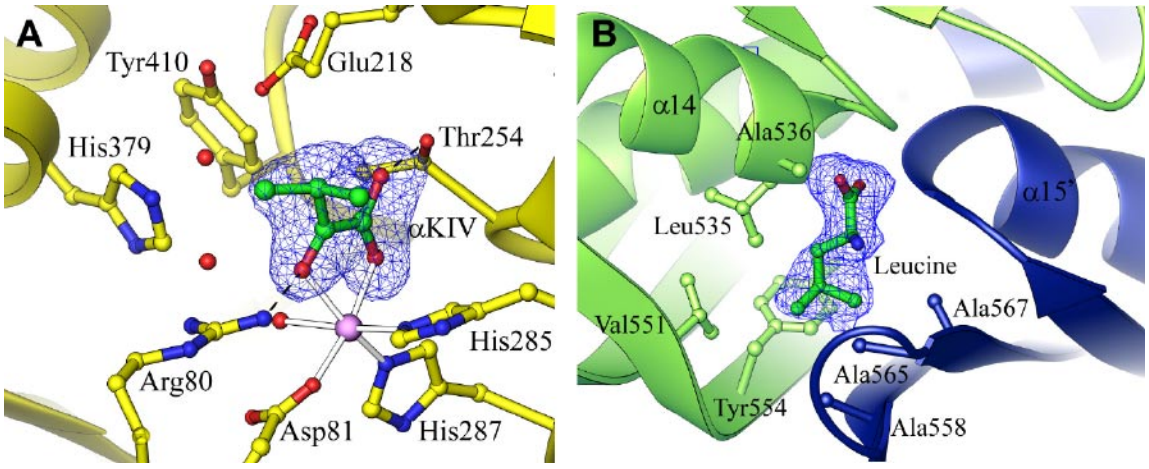


Figure 2. *Close-up of the ligand binding sites. (A) The α-KIV substrate (shown in its electron density as a blue mesh), bound to zinc. Two water molecules (small red spheres) mark where the acetyl group of acetyl-CoA would bind. The side chains of His379 and Tyr410 are from helix α10 of the other monomer, which covers the active site. (B) A leucine molecule bound in the regulatory domain with its carboxyl group between two helices.*